Table 1 Amino acid sequence of peptides hydrolyzed by human glandular kallikrein 2 (hK2)

G	K	A	R//	A	F	(SEQ ID NO: 1)
G	K	A	V	R//	Q	(SEQ ID NO: 2)
G	K	A	Y	F	M//	(SEQ ID NO: 3)
G	K	A	E	K	V//	(SEQ ID NO: 4)
G	K	A	F	R//	K//	(SEQ ID NO: 5)
G	K	A	K	P	R//	(SEQ ID NO: 6)
G	K	A	A	Y	Y//	(SEQ ID NO: 7)
G	K	A	W	Y	H//	(SEQ ID NO: 8)
G	K	A	F	R//	R//	(SEQ ID NO: 9)
G	K	A	L	Q	R//	(SEQ ID NO: 10)
G	K	A	M	R//	Q//	(SEQ ID NO: 11)
G	K	A	A	L	M//	(SEQ ID NO: 12)
G	K	A	Q	G	F//	(SEQ ID NO: 13)
G	K	A	N	M	N//	(SEQ ID NO: 14)

Random library constructed with sequence
NO_2-Y-G-K-A-X1-X2-X3-Dap-F-K(ABZ)
Where NO_2-Y is nitrotyrosine quencher;
X1-X2-X3 are random amino acids consisting
of all natural L-amino acids except cysteine (n=19);
Dap is diamnopropanoate; K(ABZ) is lysine coupled to
fluorophore aminobenzoic acid (ABZ).
HK2 cleavage sites denoted by single or double //

FIGURE 1

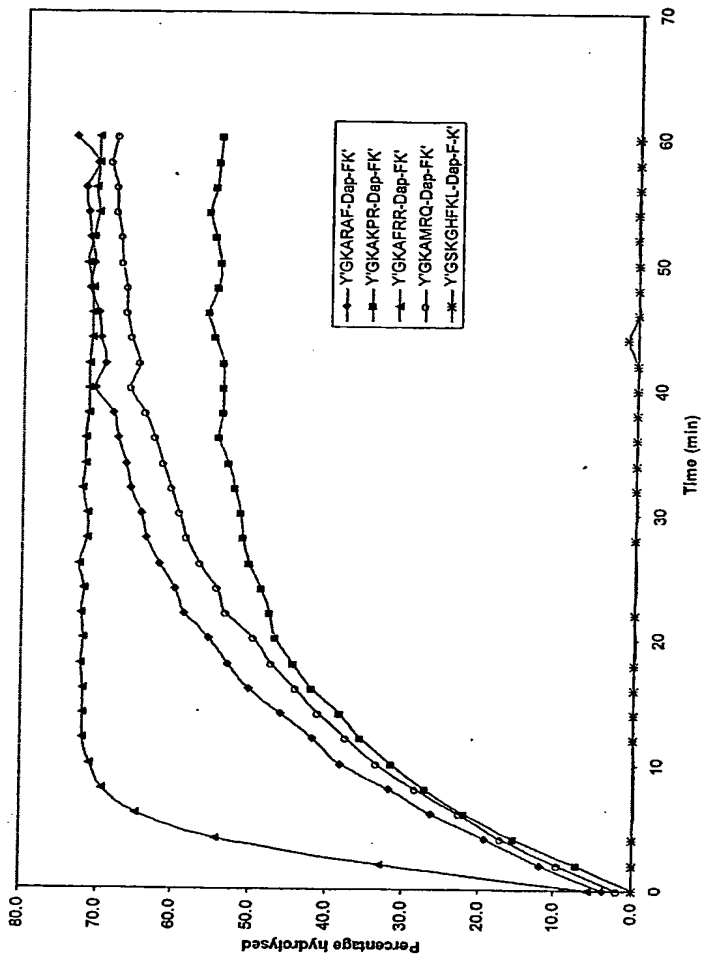

Y'GKARAF-Dap-FK'
Y'GKAKPR-Dap-FK'
Y'GKAFRR-Dap-FK'
Y'GKAMRQ-Dap-FK'
Y'GSKGHFKL-Dap-F-K'
Percentage hydrolysed
Time (min)
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0
10
20
30
40
50
60
70

Figure 2

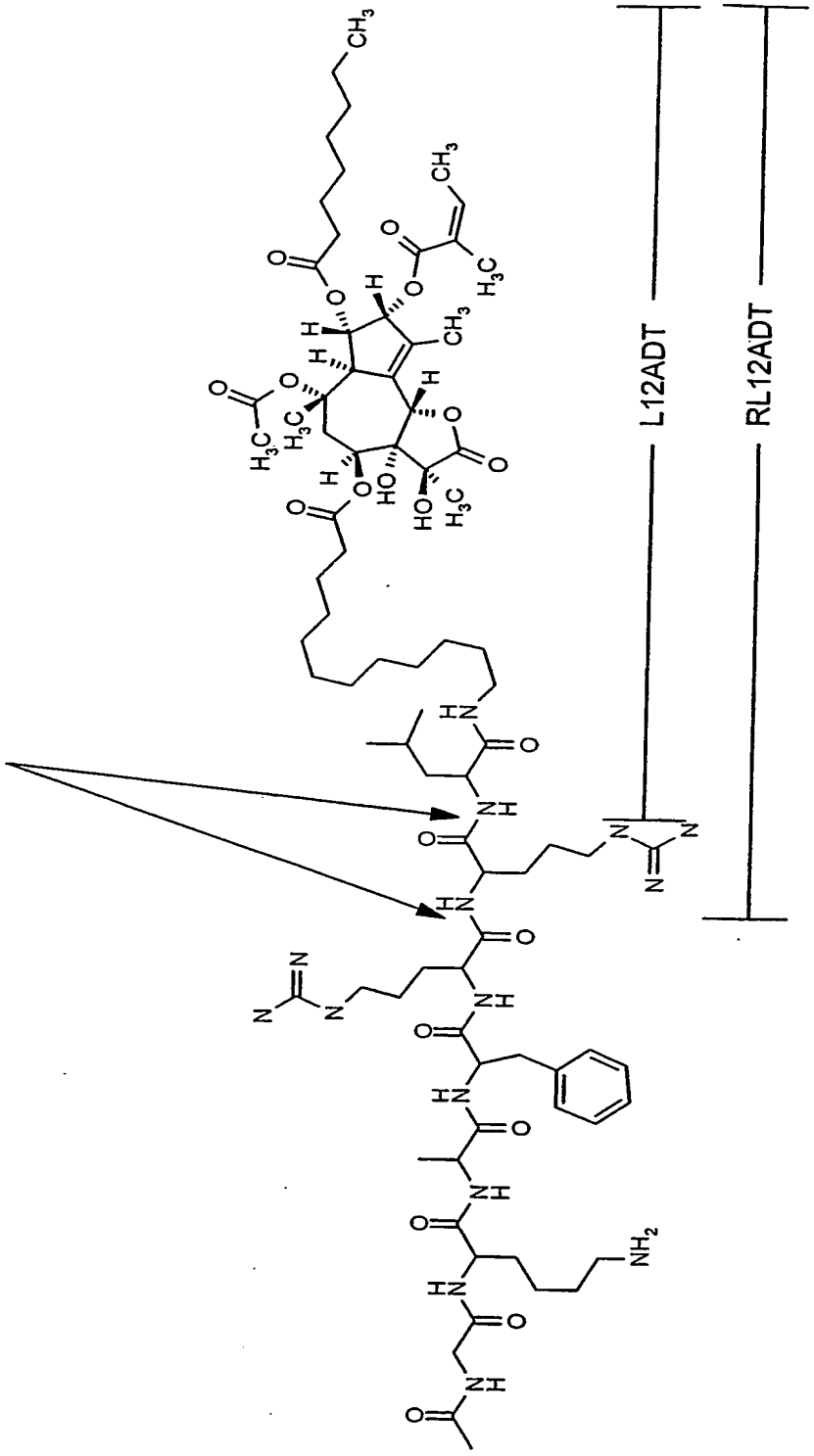
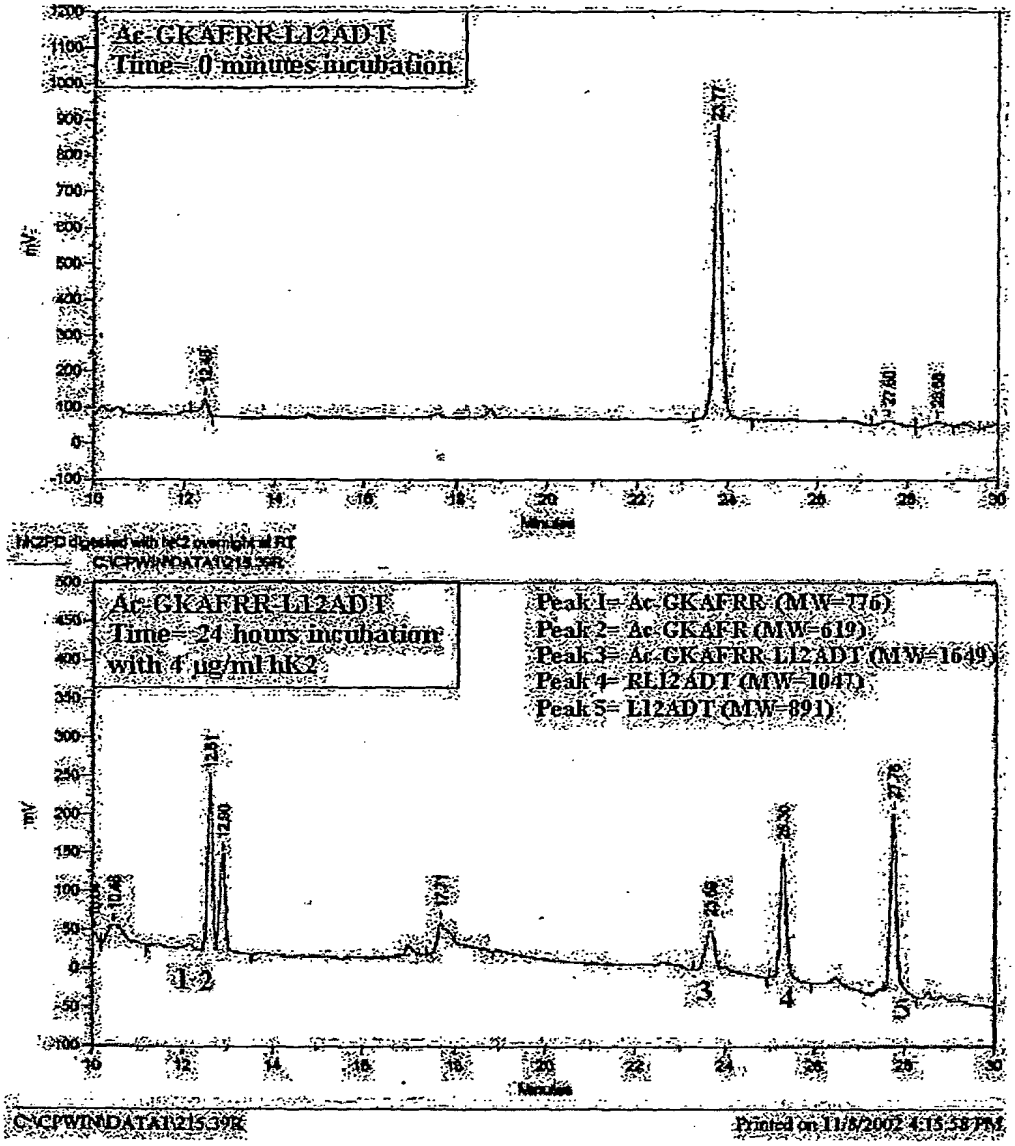
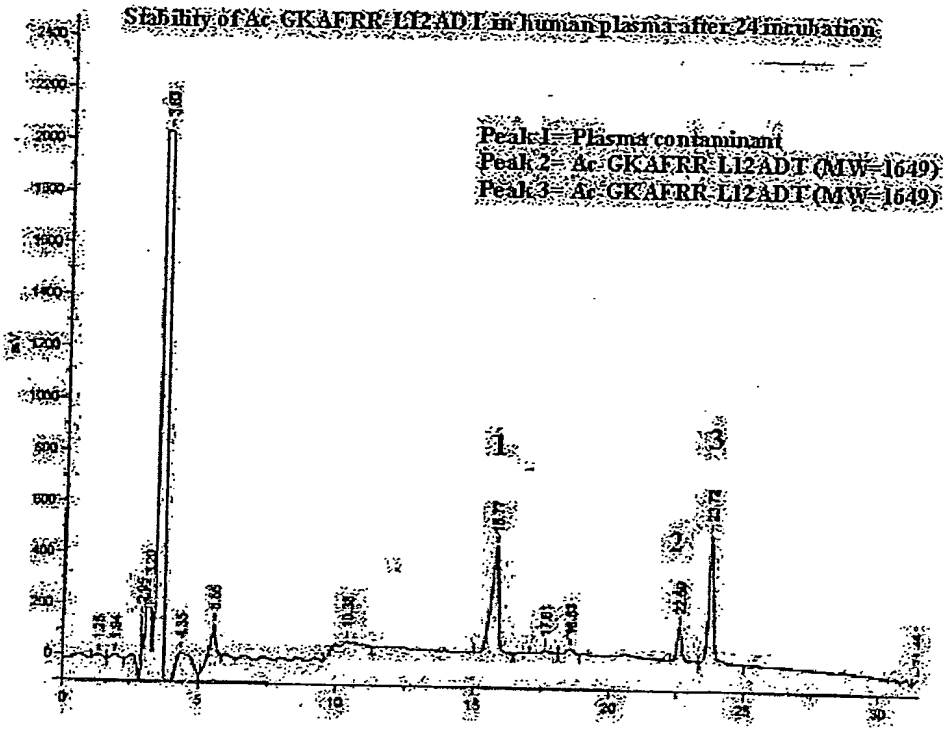


FIGURE 3



HPLC analysis of hydrolysis of hK2 prodrug Ac-GKAFRR-L12ADT by hK2 (4 µg/ml) over 24 hr incubated in 50 mM TRIS, 0.1 M NaCl, pH 7.8 at room temperature. Mass of each peak confirmed by MALDI-TOF mass spectrometric analysis (see figure 3 for mass profiles).

FIGURE 4



HPLC analysis of Ac-GKAFRR-L12ADT incubated in 50% human plasma for 24 hrs at room temperature. Peak 1 represents unidentified plasma contaminant that was also present in control plasma. Peak 2 and 3 both represent Ac-GKAFRR-L12ADT as confirmed by MALDI-TOF mass spectrometric analysis.

FIGURE 5

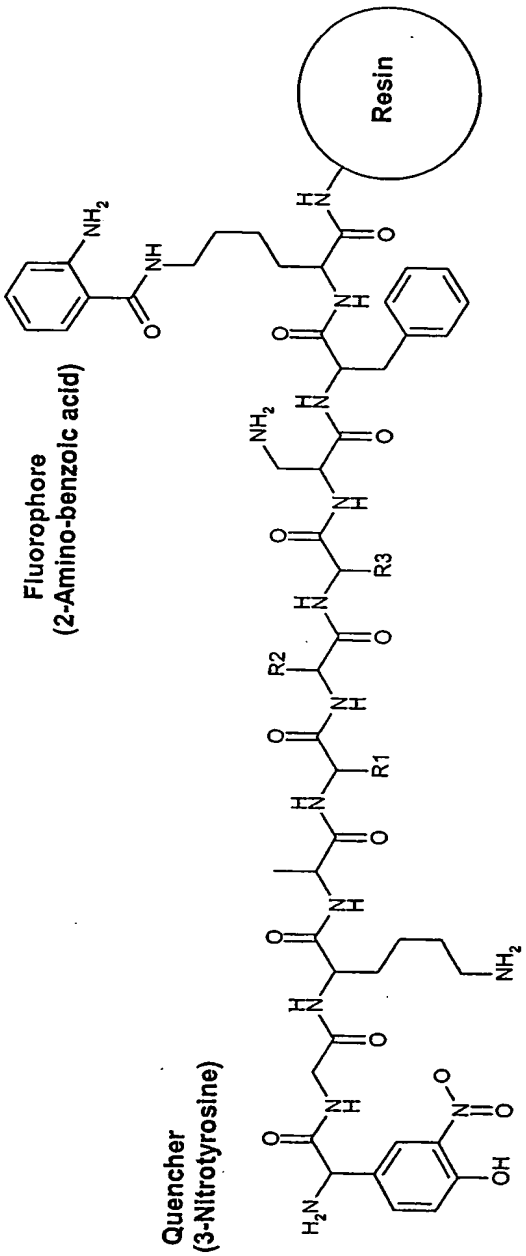

Resin
Fluorophore
(2-Amino-benzoic acid)
Quencher
(3-Nitrotyrosine)
R1
R2
R3

FIGURE 6

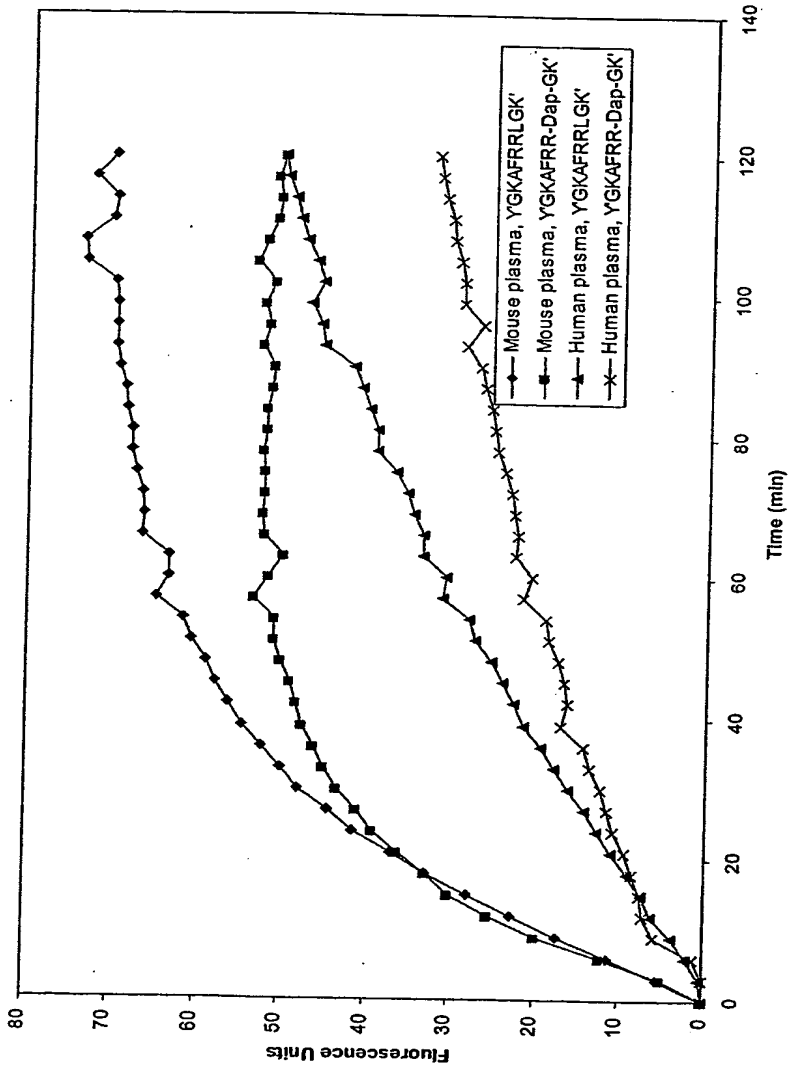

Fluorescence Units
Time (min)
Mouse plasma, YGKAFRRLGK'
Mouse plasma, YGKAFRR-Dap-GK'
Human plasma, YGKAFRRLGK'
Human plasma, YGKAFRR-Dap-GK'
0
10
20
30
40
50
60
70
80
0
20
40
60
80
100
120
140

FIGURE 7

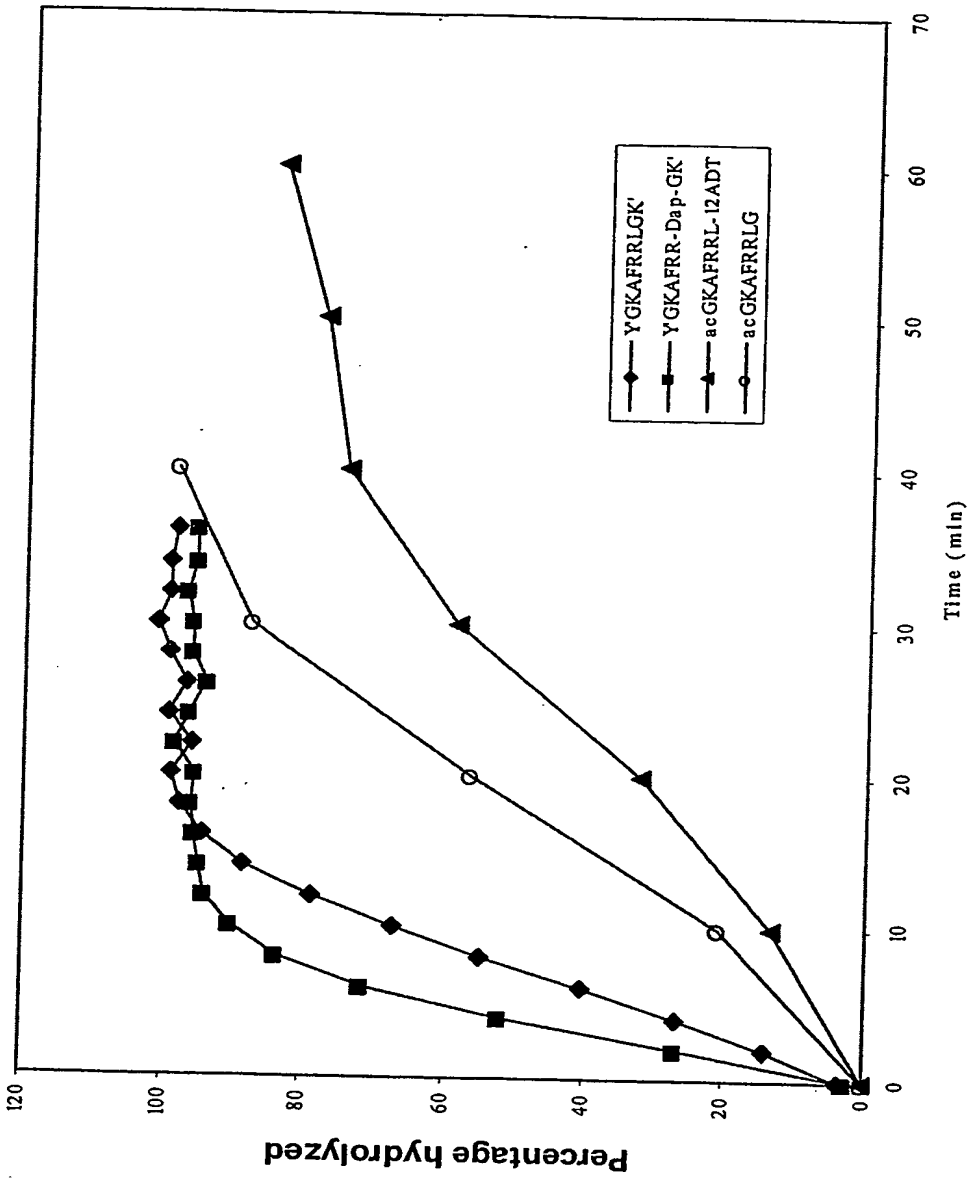

Y'GKAFRRLGK'
Y'GKAFRR-Dap-GK'
acGKAFRRL-12ADT
acGKAFRRLG
Percentage hydrolyzed
Time (min)
0
20
40
60
80
100
120
0
10
20
30
40
50
60
70

FIGURE 8

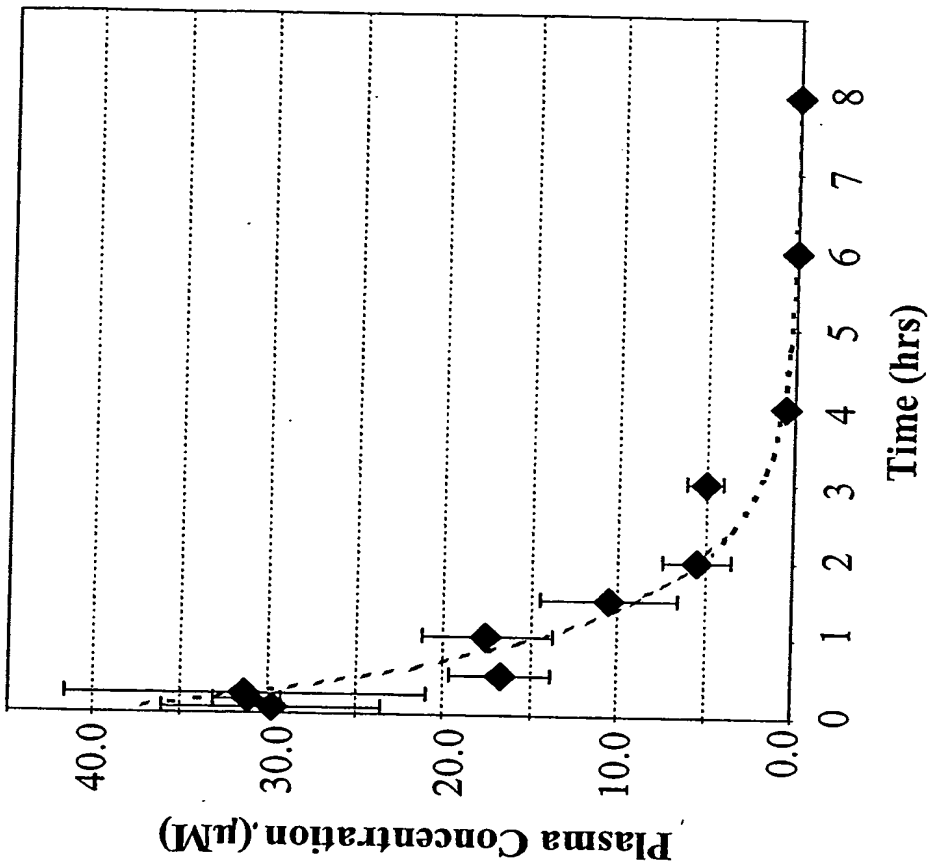

Plasma Concentration (μM)
40.0
30.0
20.0
10.0
0.0
0 1 2 3 4 5 6 7 8
Time (hrs)

FIGURE 9